Identification of *Trans*-Acting Genes Necessary for Centromere Function in *Drosophila melanogaster* Using Centromere-Defective Minichromosomes

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ABSTRACT

Deletions in the Drosophila minichromosome *Dp1187* were used to investigate the genetic interactions of *trans*-acting genes with the centromere. Mutations in several genes known to have a role in chromosome inheritance were shown to have dominant effects on the stability of minichromosomes with partially defective centromeres. Heterozygous mutations in the *ncd* and *klp3A* kinesin-like protein genes strongly reduced the transmission of minichromosomes missing portions of the genetically defined centromere, but had little effect on the transmission of minichromosomes with intact centromeres. Using this approach, *ncd* and *klp3A* were shown to require only the centromeric region of the chromosome for their roles in chromosome segregation. Increased gene dosage also affected minichromosome transmission and was used to demonstrate that the *nod* kinesin-like protein gene interacts genetically with the centromere, in addition to interacting with extracentromeric regions as demonstrated previously. The results presented in this study strongly suggest that dominant genetic interactions between mutations and centromere-defective minichromosomes could be used effectively to identify novel genes necessary for centromere function.

NORMAL chromosome segregation requires an intact centromere and proteins that interact with the centromere. Proteins necessary for centromere activity include those that localize directly to the centromere and those, such as regulatory proteins and spindle proteins, that interact functionally but indirectly with the centromere. Centromere-binding protein complexes have been isolated biochemically from *Saccharomyces cerevisiae*, immunological approaches have identified human centromere-localized proteins, and genetic screens have identified genes encoding centromere-localized proteins in *S. cerevisiae* and *Schizosaccharomyces pombe* (ALLSHIRE *et al.* 1995; reviewed in PLUTA *et al.* 1995). Genetic screens for mutations causing chromosome missegregation can potentially identify proteins that interact indirectly with the centromere, as well as proteins that localize to the centromere.

Drosophila melanogaster is well suited to the study of higher eukaryotic centromeres. It provides the diversity of chromosome cycles and cell division types seen in multicellular eukaryotes, yet is amenable to genetic, molecular and biochemical dissection. The minichromosome *Dp(1;f)1187* (*Dp1187*) has proven invaluable in dissecting centromeric heterochromatin and in defining the size and composition of a functional Drosophila centromere. *Dp1187* is a 1.3-Mb minichromosome that retains all sequences necessary for normal transmission (KARPEN and SPRADLING 1992). MURPHY and KARPEN (1995b) generated deletion derivatives of *Dp1187* and measured their transmission rates; this allowed the centromere to be localized to a 420-kb region (Figure 1).

Surprisingly little is known about the proteins required for centromere function in Drosophila. Two centromere-localized proteins, ZW10 and MEI-S332, have been identified. ZW10 protein is important for anaphase chromosome movements (WILLIAMS *et al.* 1992; WILLIAMS and GOLDBERG 1994), while MEI-S332 is necessary for sister chromatid cohesion during meiosis (KERREBROCK *et al.* 1992, 1995). Mutations affecting chromosome segregation have been recovered in screens for increased meiotic chromosome nondisjunction, or embryonic or late larval lethality (reviewed in GATTI and BAKER 1989); however, most mutations recovered in these screens do not affect the centromere. No screen in Drosophila has been designed that targets genes necessary for centromere function.

In yeasts, genes necessary for centromere function have been identified in screens for mutations that increase the instability of chromosomes with defective centromeres (DOHENY *et al.* 1993; XIAO *et al.* 1993; STRUNNIKOV *et al.* 1995). We hypothesized that a similar approach might be feasible in Drosophila using *Dp1187* deletion derivatives that have a portion of the centromere missing. We predicted that the transmission of a minichromosome with a partially functional centromere would be particularly sensitive to reduced dosage of a gene necessary for centromere function. Of particular interest are mutations with recessive effects on the

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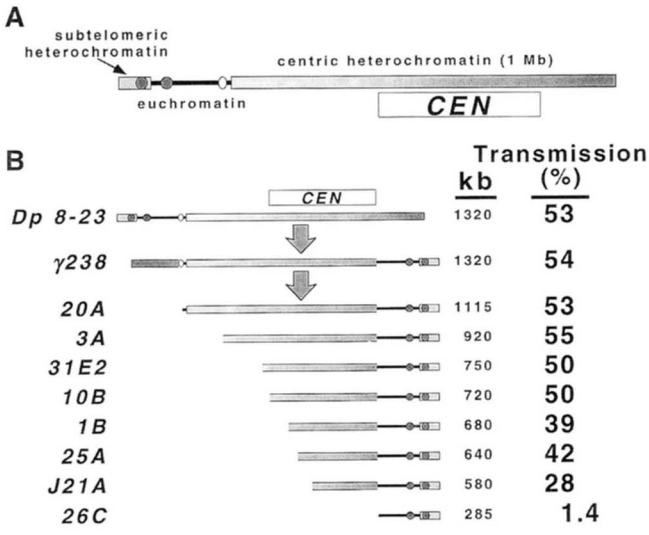

FIGURE 1.—The *Dp1187* minichromosome derivatives and centromere localization. (A) The 1.3-Mb full-length minichromosome *Dp 8-23* was derived from *Dp1187* by the insertion of two ry^+ *P* elements (gray circles) into the subtelomeric heterochromatin (light gray box) and distal euchromatin (solid line). The y^+ gene (open circle) is present in the euchromatin. The minichromosome contains 1 Mb of centric heterochromatin (gradient box) including the 420-kb centromere region (CEN). (B) The *γ238* derivative series. *γ238* was generated from *Dp 8-23* by a γ-ray-induced inversion event. γ-ray-induced terminal deletions of *γ238* that removed the y^+ gene gave rise to a series of derivatives, including the derivatives shown. Derivative sizes (kilobases) and monosome transmission rates through females are shown (MURPHY and KARPEN 1995b). The *10B* derivative shows stable transmission, and demarcates the left side of the centromere. Larger derivatives are also stable, but smaller derivatives show reduced transmission.

transmission of normal chromosomes and dominant effects on the transmission of centromere-defective minichromosomes, because these mutations should identify proteins interacting specifically with the centromere. Heterozygous mutations further destabilizing centromere-defective minichromosomes should identify both genes encoding centromere-localized proteins, such as kinetochore components and sister chromatid cohesion proteins, and genes encoding proteins that interact functionally with the centromere, such as regulatory proteins and spindle components. We endeavored to identify heterozygous mutations affecting centromere-defective minichromosome transmission, but not affecting normal chromosome transmission, as a way to identify proteins important to centromere function. This approach should identify many genes interacting with the centromere, but it cannot identify them all: mutation heterozygosity will affect transmission of a particular derivative only when it reduces gene dosage below some phenocritical threshold and when at least a portion of the chromosomal region necessary for the action of the gene has been deleted. A clear advantage of screening heterozygous mutations is that mutations deleterious to homozygotes (*e.g.*, recessive lethals and female steriles) can easily be examined for effects on centromere functions in adults.

Dp1187 derivatives can be used to determine which chromosomal regions must be present for the action of a chromosome segregation gene. MURPHY and KARPEN (1995a) demonstrated that the transmission of structurally altered minichromosomes was sensitive to reduced dosage of the *nod* kinesin-like protein gene. They found that transmission was reduced when extracentromeric regions were deleted. This agreed well with localization of NOD protein throughout chromosome arms (AFSHAR *et al.* 1995a,b) and provided evidence that NOD extracentromeric interactions are indeed essential to normal chromosome inheritance. In contrast, most loss-of-function mutations in genes needed specifically for centromere function should have strong effects on the transmission of derivatives missing portions of the centromere but should have negligible effects on the transmission of larger derivatives retaining an intact centromere.

The goal of this study was to test whether centromere-defective minichromosomes can be used to identify loci that interact genetically with the centromere. We demonstrate that heterozygous mutations in known chromosome segregation genes can destabilize centromere-defective derivatives and that these mutations interact specifically with the centromere region. This indicates that screening for interactions between heterozygous mutations and centromere-defective derivatives would be an effective method for identifying new genes necessary for centromere function. Additionally, we demonstrate that the effects of increased gene dosage on derivative transmission can provide insights into the roles of a *trans*-acting gene (*nod*) in centromere function.

MATERIALS AND METHODS

Fly strains: *Dp1187* and its derivatives are described in KARPEN and SPRADLING (1990, 1992), TOWER *et al.* (1993), LE et al. (1995) and MURPHY and KARPEN (1995b). Mutations and balancers are described in LINDSLEY and ZIMM (1992). *grau* is described in LIEBERFARB *et al.* (1996) and PAGE and ORR-WEAVER (1996). Fly stocks were gifts of S. ENDOW (*ncd*), M. GATTI (*pasc*), M. GOLDBERG [*l(1)zw10*, *rod* and *klp3A*], S. HAWLEY (*nod*) and T. ORR-WEAVER (*ord*, *mei-S332*, *grau* and *cort*). Deficiency stocks were obtained from the Bloomington Drosophila Stock Center.

Monosome transmission assays: A ry^{506} stock was constructed for each mutant chromosome tested for effects on *Dp1187* derivative transmission. Crosses of *mutation/balancer; ry/ry* females to *ry/ry;Dp* males produced *mutation/+; ry/ry* and *balancer/+; ry/ry* siblings carrying a single ry^+-marked *Dp1187* derivative (*Dp*). Monosome transmission rates were assessed in females by crossing *mutation/+; ry/ry;Dp* or *balancer/+; ry/ry;Dp* females to *YSX.YL, In(1)EN/0; ry/ry* males and scoring the percent of progeny which received the ry^+ *Dp*. The *YSX.YL, In(1)EN* chromosome ($\widehat{XY}$) suppressed poor ry^+ expression in some derivatives caused by heterochromatic position effects (KARPEN and SPRADLING 1992). Only $X/\widehat{XY}$ progeny were scored; *X*/O siblings were not scored due to enhanced ry^+ silencing. Monosome transmission rates were

assessed in males by crossing *mutation/+; ry/ry; Dp* or *balancer/+; ry/ry; Dp* males to $\widehat{XY}/\widehat{XY}; ry/ry$ females. Both $\widehat{XY}/X$ and $\widehat{XY}/Y$ progeny were scored.

Dp transmission rates were calculated as the ratio of ry^+ progeny to total progeny. A minimum of 30 progeny per female was scored. Calculating transmission rates for individual females allowed us to measure the variability between females as a standard deviation. In calculating average transmission rates, each female was given equal weight regardless of progeny number (MURPHY and KARPEN 1995a). Average transmission rates were compared using Student's *t*-test. The percent transmission drop between the first (*a*) and second (*b*) 5 days of egg lay was calculated as $[(a - b)/a] \times 100\%$. Data analyses were performed using Microsoft Excel 5.0 (Macintosh).

RESULTS

Heterozygous mutations can reduce the transmission of the centromere-defective *J21A* derivative: We hypothesized that mutations that further destabilize centromere-defective chromosomes would identify genes necessary for centromere function. This led us to test mutations for effects on the transmission of *J21A*, a *Dp1187* derivative with approximately one-third of the centromere deleted (Figure 1B). *J21A* is not transmitted to 50% of progeny as expected for a chromosome with a fully functional centromere: in our standard $y^1; ry^{506}$ genetic background, it is transmitted to 28 ± 12% of the progeny of females in the first 5 days of egg lay (first subculture), and to 22 ± 11% of progeny in the second 5 days of egg lay (second subculture) (Table 1; MURPHY and KARPEN 1995b). This monosome transmission assay measures the combined efficiency of derivative inheritance through preblastoderm and germ line mitoses and meiosis in the parent and somatic mitoses in progeny. Decreased transmission could result from instability in a single division or in many different divisions. The 21% drop in *J21A* transmission between first and second subcultures {21% = [(28 − 22%)/28%] × 100%} probably reflects chromosome loss in oogonial mitoses, because *J21A* instability in germ line stem cell divisions would be seen as declining transmission with continued egg lay.

Previously isolated recessive mutations causing missegregation of normal chromosomes seemed likely to include mutations affecting centromere function, so we chose a sample of these to test for heterozygous effects on *J21A* transmission. To judge the effects of mutations on *J21A* transmission, we compared transmission rates in heterozygous females with the rate in *y; ry* females (Table 1). Balancer chromosomes had effects on *J21A* transmission (see below), therefore *balancer/+* siblings were not used as controls. *J21A* transmission rates are highly variable in both the standard and mutant backgrounds, and this is reflected in large standard deviations. The 99% confidence interval for *J21A* transmission in the standard *y; ry* background is 24–32%; we accepted this range as typical, though other "wild-type" backgrounds might give slightly different confidence intervals.

Strong decreases in *J21A* transmission were seen in *nonclaret disjunctional* (*ncd*) and *kinesin-like protein 3A* (*klp3A*) heterozygotes. MURPHY and KARPEN (1995a) also saw a strong decrease in *nod* heterozygotes. All three loci encode kinesin-like proteins (ZHANG *et al.* 1990; HATSUMI and ENDOW 1992; WILLIAMS *et al.* 1995). The ncd^1 chromosome reduced transmission to 19% (*vs.* 28%, $P < 10^{-4}$) in the first subculture and 15% (*vs.* 22%, $P < 0.004$) in the second subculture. Three *klp3A* mutations decreased first subculture transmission to 7, 9 and 16% ($P < 10^{-6}$), and $klp3A^{835}$ decreased second subculture transmission to 1%. A drop in *J21A* transmission of ~20% between subcultures in wild-type females suggests that *J21A* is usually unstable in oogonial mitoses. The much larger transmission drop between subcultures in $klp3A^{835}$ heterozygotes (86%) suggests enhanced instability in oogonial divisions and provides another criterion for judging mutant effects. The *klp3A* alleles were generated in two different parental chromosomes (WILLIAMS *et al.* 1995), which strongly suggests that the *klp3A* mutations, and not linked modifiers, are responsible for the transmission effects.

Weak decreases in *J21A* transmission were seen in females heterozygous for a chromosome with *mei-S332* and *orientation disruptor* (*ord*) mutations. First subculture transmission in $mei\text{-}S332^1$ $ord^1/+$ females was 24%, a value little different from 28% ($P \leq 0.17$), but second subculture transmission dropped to 14%, a value significantly lower than 22% ($P < 10^{-3}$). The 42% drop in transmission between subcultures further indicates weak effects on *J21A* transmission. A weak effect was also seen in ord^1 heterozygotes, where transmission between subcultures dropped 34%. Since the stronger ord^2 and ord^8 mutations (MIYAZAKI and ORR-WEAVER 1992) had no effects, modifiers on the ord^1 and $mei\text{-}S332^1$ ord^1 chromosomes may be responsible for transmission decreases, rather than the ord^1 mutation. The *ord* and *mei-S332* loci are required for sister chromatid cohesion in meiosis, and *ord* is also required for proper chromosome disjunction in germ line mitoses (LIN and CHURCH 1982; KERREBROCK *et al.* 1992; MIYAZAKI and ORR-WEAVER 1992). While these experiments demonstrate that weak effects of chromosome substitutions on *J21A* transmission can be recognized, they also illustrate why it is important to show that transmission effects are caused by the named mutation rather than linked modifiers.

Although transmission rates were slightly increased in *mei-S332* heterozygotes, we suggest that these modest changes represent background variability in wild-type transmission rates rather than effects of the mutations. Overall, slight transmission increases were more common than slight decreases, which suggests that the wild-type range of *J21A* transmission rates includes values higher than the standard 28% seen in *y; ry* females.

TABLE 1

***J21A* transmission from heterozygous mutant females**

Mutant chromosome	First 5 days of egg lay		Second 5 days of egg lay		Percent transmission drop between subcultures[d]
	Percent transmission[a]	n[b]	Percent transmission[c]	n[b]	
		Standard genetic background			
y;ry	28 ± 12	53	22 ± 11	54	21
		Strong reductions			
nod^{h17}	3 ± 3*[e]	23[e]			
ncd^{1}	19 ± 7*	26	15 ± 8*	21	21
$klp3A^{E4}$	16 ± 5*	11			
$klp3A^{835}$	7 ± 5*	16	1 ± 2*	15	86
$klp3A^{1611}$	9 ± 8*	8			
		Weak reductions			
$mei\text{-}S332^{1}$ ord^{1}	24 ± 13	26	14 ± 10*	31	42
ord^{1}	29 ± 8	27	19 ± 11	25	34
		No effects			
ord^{2}	31 ± 10	25	24 ± 9	25	23
ord^{3}	23 ± 8	49	23 ± 6	42	0
$mei\text{-}S332^{1}$	35 ± 9†	29	30 ± 11†	29	14
$mei\text{-}S332^{3}$	36 ± 10†	26	29 ± 7†	25	19
$mei\text{-}S332^{6}$	33 ± 9	18	30 ± 12†	30	9
$grau^{QF31}$	24 ± 9	25	21 ± 8	29	13
$cort^{QW55}$	24 ± 9	18			
$pasc^{C204}$	27 ± 12	8			
$l(1)zw10^{S1}$	32 ± 10	11	27 ± 9	10	16
		Balancer heterozygotes			
Pooled *FM7a/+* sibs	21 ± 12	22	21 ± 15	9	0
Pooled *SM1/+* sibs	33 ± 9†	174	30 ± 9†	134	9
Pooled *TM3/+* sibs	39 ± 9†	40	36 ± 9†	22	8
FM7c/+	33 ± 14	8			
		Weak increase			
$rod^{H4.8}$	38 ± 10†	15			

* $P \leq 0.01$ for transmission decreases; † $P \leq 0.01$ for transmission increases.

[a] *t*-tests for differences from 28 ± 12%.

[b] n = number of female parents tested.

[c] *t*-tests for differences from 22 ± 11%.

[d] Calculated as [(percent transmission in first 5 days of egg lay − percent transmission in second 5 days of egg lay)/(percent transmission in first 5 days of egg lay)] × 100%.

[e] Data from MURPHY and KARPEN (1995a).

There is no evidence for dominant effects of *grauzone* (*grau*), *cortex* (*cort*), *l(1)zw10* or *parallel sister chromatids* (*pasc*) mutations on *J21A* transmission: heterozygosity for $grau^{QF31}$, $cort^{QW55}$, $l(1)zw10^{S1}$ or $pasc^{C204}$ did not significantly alter *J21A* transmission at $P \leq 0.01$. These loci may not interact with heterochromatic regions in *J21A*; other regions of the centromere (to the right of the *J21A* breakpoint) may need to be eliminated to observe an interaction, or these mutations may not reduce protein levels enough to destabilize *J21A*.

These results demonstrate that mutations with recessive effects on chromosome segregation—like *ncd*, *klp3A* and *nod*—can have strong dominant effects on the transmission of a centromere-defective minichromosome. Interactions between heterozygous mutations and the sensitized *J21A* derivative provide a valuable

new genetic assay for identifying genes necessary for chromosome inheritance.

Balancer chromosomes affect *J21A* transmission: Balancer heterozygosity had marked effects on *J21A* transmission (Table 1). Sibling females of mutation heterozygotes transmitted *J21A* at 33% for *SM1/+* ($P < 0.01$), 39% for *TM3/+* ($P < 10^{-7}$), and 21% for *FM7a/+* ($P < 0.02$). Decreased transmission in *FM7a/+* females was due to the presence of a modifier locus and not due to inversion heterozygosity. *FM7a* failed to complement the loss-of-function nod^{DR3} mutation, causing a threefold increase in chromosome *4* nondisjunction compared to $nod^{DR3}/+$ (15 *vs.* 4%), and the effect of *FM7a* on the transmission of other derivatives resembles that of nod^{b17} (see below). A heterozygous *FM7c* balancer with the same inversion breakpoints (LINDSLEY and ZIMM 1992) had nearly wild-type *J21A* transmission (33%), complemented nod^{DR3} for chromosome *4* nondisjunction (3%), and did not affect the transmission of other derivatives in the manner of nod^{b17}. This *FM7a* chromosome was derived from a *FM7a* stock characterized by ZHANG *et al.* (1990) and RASOOLY *et al.* (1991) as having wild-type *nod* function with respect to normal chromosome segregation, yet our results indicate that this *FM7a* chromosome carries a weak *nod* mutation or a mutation at a second locus that interacts with *nod*. *FM7a* may have acquired genetic changes in the interim. Alternatively, the existence of hitherto unrecognized genetic modifiers was revealed by the sensitive minichromosome transmission assays (with fourth chromosome nondisjunction being enhanced by changes in genetic background). These results suggest that transmission effects of other balancers also may be due to allelic variants rather than inversion heterozygosity.

Genetic modifiers can increase *J21A* transmission: In addition to mutations decreasing *J21A* transmission, we identified two modifiers that increase transmission. In constructing *ry* stocks for a number of *X*-linked mutations, a *FM6/y sn* $Cp36^{n1}$; *ry/ry* stock was used as the source of the *X* balancer and *ry* chromosomes. All of these stocks had very high *J21A* transmission: three different *pasc* stocks, a *l(1)zw10* stock and a number of *X* chromosome deficiency stocks had >40% transmission for both mutation and balancer heterozygotes. Further crosses mapped the "high" modifier common to all these stocks to the third chromosome from the *FM6/y sn* $Cp36^{n1}$; *ry/ry* stock. When females heterozygous for this "high" *ry* chromosome and a normal "low" *ry* chromosome from the standard *y; ry* background were mated to *y; ry; J21A* males, the transmission rates in their *J21A* female progeny showed a bimodal distribution with one peak centered at 27% and the other peak centered at 46%. These results indicate that the "high" modifier is dominant and not present in our standard *y; ry* strain and highlight the care that one must take when establishing mutant stocks to test in this sensitive assay. Mutant chromosomes should be substituted into a standard, well-characterized genetic background before testing for effects on derivative transmission.

Mutant *rough deal* (*rod*) chromosomes increased *J21A* transmission to 38% ($P \leq 0.001$) (Table 1). This increase was seen with four different *ry-*$rod^{H4.8}$ crossover chromosomes; however, additional experiments are needed to determine whether the increase is due to the *rod* mutation or to a linked modifier similar to the "high" modifier described above. Homozygous *rod* mutations are late larval lethals causing incomplete sister chromatid separation during mitosis (KARESS and GLOVER 1989). *J21A* transmission may be increased because the tendency of *J21A* to display aberrant sister chromatid cohesion (K. R. COOK and G. H. KARPEN, unpublished observations) is counteracted by the *rod* mutation.

Screening *X* deficiencies for novel chromosome transmission genes: To test whether interactions between heterozyous mutations and centromere-defective derivatives could be used to identify new transmission genes, we screened a collection of *X* chromosome deficiencies for effects on *J21A* transmission. A balanced *ry* stock was constructed for each deficiency using either *FM7a* or *FM6* (the *FM7a/y; ry/ry* stock used in deficiency stock construction was generated by substituting *FM7a* into the standard *y; ry* stock). Since the *FM6* stocks contained the third chromosome "high" modifier, the *FM7a* and *FM6* stocks will be considered separately (Table 2).

Sixteen *Df/FM7a; ry/ry* stocks were tested, which together uncover 30% of the *X* chromosome. Only *Df(1)sd72* decreased *J21A* transmission significantly (21%, $P \leq 0.004$). There is no known chromosome segregation gene in the region of this deficiency; further dissection of this region will allow the specific dosage-sensitive transmission gene(s) to be identified. This result strongly suggests that dose-dependent loci with statistically significant effects on *J21A* transmission ($P < 0.01$) can be detected by screening deficiencies, and demonstrates that loci with moderately strong effects are not present in most chromosomal regions. On the other hand, loci with very weak effects may be common: five deficiencies (*Df(1)sd72b, Df(1)N19, Df(1)RK4, Df(1)4b18* and *Df(1)JC70*) gave *J21A* transmission rates that were statistically significant at much lower confidence levels ($0.05 < P < 0.20$).

Ten *Df/FM6; ry/ry* stocks were tested. Transmission in *Df/+* females was compared with transmission in their *FM6/+* siblings (39%). Two deficiencies decreased *J21A* transmission. *Df(1)HA85* transmitted *J21A* at 16% ($P \leq 0.001$), and this rate is low enough to be significant in either the *FM6* or *FM7a* background. *Df(1)HA85* uncovers the *nod* locus. Since the amorphic nod^{b17} allele gave 3% transmission in the standard *y; ry* background, the third chromosome "high" modifier probably counteracts the reduction in transmission

TABLE 2

***J21A* transmission from females heterozygous for *X* chromosome deficiencies**

Deficiency	Breakpoints	Percent transmission	n^a
	FM7 stocks[b]		
Df(1)sd72b	13F1;14B1	21 ± 7*	18
Df(1)N19	17A1;18A2	21 ± 9	9
Df(1)RK4	12F5-6;13A9-B1	23 ± 8	9
Df(1)4b18	14B8;14C1	23 ± 9	8
Df(1)dm75e19	3C11;3E4	24 ± 6	8
Df(1)RK2	12D2-E1;13A2-5	25 ± 14	7
Df(1)JA27	18A5;20A	25 ± 11	12
Df(1)sqh	5D1-2;5E	26 ± 12	12
Df(1)JC4	20A1;20E-F	27 ± 13	17
Df(1)N12	11D1-2;11F1-2	28 ± 12	6
Df(1)B	16A2;16A6	28 ± 10	4
Df(1)N73	5C2;5D5-6	29 ± 9	10
Df(1)JF5	5E3-5;5E8	30 ± 4	14
Df(1)HA32	6E4-5;7A6	30 ± 13	8
Df(1)C149	5A8-9;5C5-6	30 ± 8	8
Df(1)JC70	4C15-16;5A1-2	33 ± 9	5
	FM6 stocks[c]		
Df(1)HA85	10C1-2;11A1-2	16 ± 13*	15
Df(1)sqh	5D1-2;5E	28 ± 10*	13
Df(1)JA27	18A5;20A	34 ± 8	7
Df(1)JC19	2F6;3C5	36 ± 12	8
Df(1)N19	17A1;18A2	18 ± 8	7
Df(1)KA14	7F1-2;8C6	39 ± 7	16
Df(1)g	11F10; 12F1	41 ± 5	12
Df(1)C149	5A8-9;5C5-6	42 ± 7	11
Df(1)RK2	12D2-E1;13A2-5	44 ± 6	8
Df(1)JF5	5E3-5;5E8	47 ± 10†	19
Pooled *FM6/+* sibs		39 ± 9	80

* $P \leq 0.01$ for transmission decreases.
† $P \leq 0.01$ for transmission increases.
[a] n = number of female parents tested.
[b] t-tests for differences from 28 ± 12%.
[c] t-tests for increases or decreases from pooled *FM6/+* sibs.

caused by *nod* heterozygosity. *Df(1)sqh* transmitted *J21A* at 28% ($P < 0.001$), but it did not cause a large decrease in the *FM7a* background. *Df(1)JF5* increased transmission in the "high" background, though it showed normal transmission in the *FM7a* background. We conclude that the interactions of deficiencies and the "high" modifier are potentially complex, and that the effects of some deficiencies could be masked in this background. This again highlights the importance of a defined genetic background when testing mutations in this sensitive assay.

These results show that heterozygous deficiencies can have strong effects on the transmission of a centromere-defective minichromosome and indicate that the *J21A* transmission assay can be used to identify novel dose-dependent loci. Furthermore, these results indicate that loci with strong dosage-sensitive interactions are not so common as to be encountered in most chromosomal intervals.

***Trans*-acting genes can interact specifically with the centromere:** The regions of *Dp1187* that are required for the action of a gene in chromosome segregation can be identified and mapped using the series of *γ238* terminal deletion derivatives (Figure 1B; MURPHY and KARPEN 1995a). We predicted that mutations in genes needed specifically for centromere function would strongly decrease the transmission of derivatives that lack portions of the 420-kb fully functional centromere, but would have little effect on the transmission of derivatives with an intact centromere. The effects of ncd^1, $klp3A^{835}$ and $mei\text{-}S332^1$ ord^1 mutant chromosomes on the transmission of *γ238* derivatives confirmed that some mutations interact specifically with the centromere (Figure 2). The transmission of derivatives with partially functional centromeres—*1B*, *25A* and *J21A*—showed the greatest sensitivity to these heterozygous chromosomes, while derivatives with intact centromeres (*10B* and larger) showed only modestly reduced transmis-

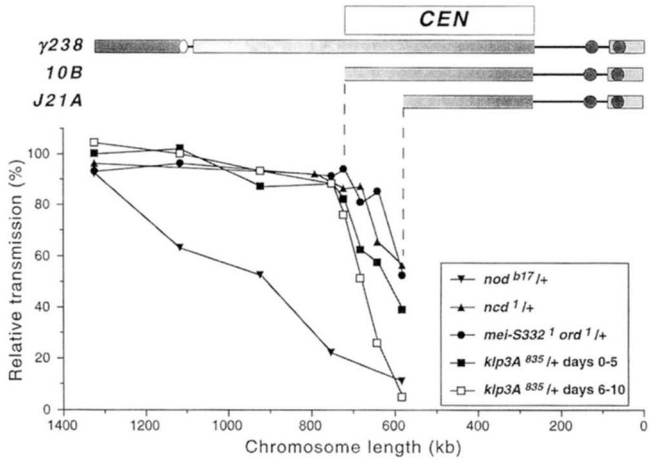

FIGURE 2.—Transmission of *γ238* derivatives in the presence of heterozygous *ncd*^{1}, *klp3A*^{835}, *mei-S332*^{1} *ord*^{1} and *nod*^{b17} chromosomes shown relative to derivative transmission in the *y; ry* background. Heterozygous females were progeny of the cross *mutation/balancer* (*ry/ry*) females × *y; ry; Dp, y*$^{+}$ males. All transmission rates are shown relative to transmission in *y; ry; Dp, ry*$^{+}$ females for days 0–5 of egg lay; standard *y; ry* relative transmission is 100% for each derivative. Day 0–5 transmission is shown for *nod* heterozygotes (data from MURPHY and KARPEN 1995a), and day 6–10 transmission is shown for *ncd*^{1} and *mei-S332*^{1} *ord*^{1} heterozygotes. Transmission in *klp3A* heterozygotes is shown for both days 0–5 and days 6–10.

sion. The smallest derivative with an intact centromere, *10B*, was weakly sensitive to the effects of *klp3A*. The effects of the *ncd*^{1}, *klp3A*^{835} and *mei-S332*^{1} *ord*^{1} mutant chromosomes varied in strength with *klp3A* having the strongest effect.

The pattern of centromere-specific interactions seen with *ncd*, *klp3A* and *mei-S332 ord* mutant chromosomes is in marked contrast to the pattern seen in *nod* heterozygotes, where derivatives missing extracentromeric regions were destabilized (Figure 2; MURPHY and KARPEN 1995a). The two patterns could reflect qualitatively different genetic interactions or the *nod* pattern could represent a quantitatively more severe version of the centromere-specific pattern. The interactions of *klp3A* demonstrate that the difference is indeed qualitative. Transmission of the *1B*, *25A* and *J21A* centromere-defective derivatives decreased substantially in the second subculture of the *klp3A* heterozygotes described above, while the transmission of larger, stable derivatives was nearly unchanged (Figure 2). *J21A* transmission decreased below the level seen even in *nod* heterozygotes.

These results demonstrate that genes necessary for chromosome segregation can interact specifically with the centromere. The ability to map chromosomal interaction regions using *Dp1187* deletion derivatives enables us to distinguish two classes of chromosome transmission genes. One class, which includes *klp3A* and *ncd*, requires only centromeric sequences for its action in chromosome segregation, while the other class, currently defined only by *nod*, requires extracentromeric regions for its action.

Increased *nod*$^{+}$ gene dosage rescues the transmission of centromere-defective derivatives: The strong effects of reduced gene dosage on transmission of centromere-defective derivatives suggested that adding copies of a gene necessary for centromere function might compensate for deleting a portion of the centromere. Increased gene dosage could be used to determine the chromosomal regions necessary for the action of a gene in chromosome segregation. The existence of *nod*$^{+}$ interaction sites within the centromere, in addition to those in extracentromeric regions, was suggested by MURPHY and KARPEN (1995a), but could not be conclusively shown from the effects of reduced *nod*$^{+}$ gene dosage on derivative transmission. We used the effects of increased *nod*$^{+}$ dosage on *Dp1187* derivative transmission to examine *nod* interactions with the centromere.

We used a *nod*$^{+}$ *P*-element transgene (AFSHAR *et al.* 1995b; AFSHAR 1996) to introduce one or two extra copies of *nod*$^{+}$ to assess the effects of increased *nod*$^{+}$ dosage on centromere function. *J21A* transmission in *nod* heterozygotes was partially rescued by copies of the *nod*$^{+}$ transgene (Table 3): transmission increased from 3 to 7% ($P < 0.001$) with one copy of the transgene, and increased to 11% with two copies (*vs.* 7%, $P < 0.01$). Transmission did not reach the level seen in *nod*$^{+}$ homozygotes, strongly suggesting that the transgene is not expressed as highly as the endogenous gene. Similarly, two transgene copies did not increase *10B* transmission to normal levels in the presence of one endogenous *nod*$^{+}$ gene.

When *nod*$^{+}$ transgene copies were added to two endogenous *nod*$^{+}$ copies, the transmission of centromere-defective derivatives increased. The addition of one and two copies of the *nod*$^{+}$ transgene in *nod*$^{+}$ homozygotes increased *J21A* transmission from 24 to 31% ($P < 0.02$) and 36% ($P < 0.01$), respectively. These observations show that the amount of NOD in wild-type females limits the transmission of *J21A*, and that insufficient *nod*$^{+}$ interactions contribute substantially to reduced *J21A* transmission. The transmission of *10B*, a derivative with an intact centromere, is normal in the presence of two endogenous *nod*$^{+}$ genes and was unaffected by *nod*$^{+}$ transgene copies. These results suggest that *nod*$^{+}$ interacts with the 140-kb chromosomal region between the *J21A* and *10B* breakpoints; thus, a *nod*$^{+}$ interacting region coincides with a portion of the genetically defined centromere.

Interestingly, the transmission of *26C*, a derivative missing all centric heterochromatin and retaining only euchromatic sequences and subtelomeric heterochromatin (MURPHY and KARPEN 1995b), improved in the presence of increased *nod*$^{+}$ dosage: *26C* transmission in *nod*$^{+}$ homozygotes increased from 3 to 5% ($P < 0.06$) with one transgene copy and 9% ($P < 0.01$) with two transgene copies. Recent studies (B. C. WILLIAMS, T. D. MURPHY, M. L. GOLDBERG and G. H. KARPEN, unpublished data) suggest that structurally "acentric" deriva-

TABLE 3

The effect of *nod*$^+$ dosage on derivative transmission

Genotype	Endogenous *nod*$^+$ copies	Transgene *nod*$^+$ copies	*10B* Percent transmission	*10B* n^a	*J21A* Percent transmission	*J21A* n^a	*26C* Percent transmission	*26C* n^a
nod$^-$/*nod*$^+$ [b]	1	0			3 ± 2	23	1 ± 1	16
nod$^-$/*nod*$^+$;*P*[*nod*$^+$]	1	1	16 ± 6	20	7 ± 6†††	35	1 ± 1	21
nod$^-$/*nod*$^+$;*P*[*nod*$^+$]/*P*[*nod*$^+$]	1	2	18 ± 8	17	11 ± 5†††	22	1 ± 1	14
nod$^+$/*nod*$^+$	2	0	48 ± 5†††	14	24 ± 8†††	12	3 ± 3†††	26
nod$^+$/*nod*$^+$;*P*[*nod*$^+$]	2	1	46 ± 8	49	31 ± 10††	65	5 ± 4†	66
nod$^+$/*nod*$^+$;*P*[*nod*$^+$]/*P*[*nod*$^+$]	2	2	48 ± 8	20	36 ± 10†††	36	9 ± 7††	23

Progeny from crosses *y nod*$^+$/*y nod*$^+$;+/+; *ry/ry;Dp, ry*$^+$ females × *y nod*$^+$/*Y;Sp/P*[*nod*$^+$];*ry/ry* males and *y nod*^{h17} or *y nod*$^+$/*y nod*$^+$;*P*[*nod*$^+$]/*SM1;ry/ry* females × *y nod*$^+$/*Y;P*[*nod*$^+$]/*Sp;ry/ry;Dp, ry*$^+$ males. Rates in *SM1* progeny are not reported. *P* value given for significant increase from transmission rate in the immediately preceding row. $^{\dagger\dagger\dagger}P \leq 0.01$, $^{\dagger\dagger}0.01 < P \leq 0.05$, $^{\dagger}0.05 < P \leq 0.10$ for transmission increases.

[a] *n* = number of female parents tested.

[b] Data from MURPHY and KARPEN (1995a).

tives like *26C* have acquired neocentromeric activity, *i.e.* sequences that do not normally function as centromeres are capable of nucleating a kinetochore-like structure. Our observations suggest that neocentromeres, like conventional centromeres, are stabilized by *nod* interactions.

In conclusion, the *nod*$^+$ transgene was able to partially rescue the transmission of *J21A* and *26C*, demonstrating that increased gene dosage, as well as reduced gene dosage, can affect the transmission of *Dp1187* derivatives. These observations suggest the existence of *nod* interacting regions within the 420-kb genetically defined centromere in addition to those mapped previously to extracentromeric regions of *Dp1187* (MURPHY and KARPEN 1995a).

The transmission of *J21A* in heterozygous mutant males: Centromere-defective derivatives are transmitted at higher rates in males than in females (MURPHY and KARPEN 1995b); *J21A* is transmitted at 40% in males *vs.* 28% in females. *Trans*-acting genes may interact differently with the centromere in the two sexes or different sets of genes may be active. We examined male *J21A* transmission in the presence of heterozygous mutations in chromosome segregation genes. Mutations in *nod* and *ncd* were not examined because homozygous mutant males display no aberrant phenotype (DAVIS 1969; CARPENTER 1973; ZHANG and HAWLEY 1990); likewise, *klp3A, l(1)zw10* and *pasc* mutations could not be examined because they are *X*-linked lethals or male steriles. The *mei-S332*^{1} *ord*^{1} chromosome, which reduced female *J21A* transmission, and the *rod* chromosome, which increased female *J21A* transmission, had no effects in males (Table 4). *ord* and *mei-S332* mutant chromosomes had no effects on female *J21A* transmission and also had no effects in males [*J21A* transmission in *mei-S332*^{3}/+ males was significantly lower than in *SM1*/+ siblings ($P \leq 0.01$), but was not significantly lower than in *y;ry* males ($P \leq 0.40$)]. Homozygous *grau* mutations do not affect males (PAGE and ORR-WEAVER 1996) and, as expected, *grau*^{QF31}/+ had no effect. The apparent greater sensitivity of *J21A* transmission to mutations in females suggests that screening for genes necessary for centromere function using centromere-defective derivatives would be more efficient in females; however, screening in males might identify genes expressed differently or specifically in males.

DISCUSSION

In this study, we used centromere-defective deletion derivatives of the *Dp1187* minichromosome to demonstrate that *trans*-acting genes can interact specifically with the centromere. Heterozygous mutations in genes necessary for centromere function destabilized deriva-

TABLE 4

Transmission of *J21A* from heterozygous mutant males

Genotype	Percent transmission [a]	n^b
y;ry	40 ± 6	59
grau^{QF31}	41 ± 6	15
ord^1	41 ± 9	23
ord^2	46 ± 9	19
ord^3	44 ± 8	26
mei-S332^1	42 ± 6	24
mei-S332^3	39 ± 5*	26
mei-S332^6	45 ± 8	24
mei-S332^1 *ord*^{1}	38 ± 12	15
Pooled *SM1*/+ sibs	42 ± 7	171
rod$^{114.8}$	40 ± 7	41
TM3/+ sibs	45 ± 9	35

* $P \leq 0.01$ for transmission decreases.

[a] *t*-tests for increases or decreases from pooled *SM1*/+ sibs or *TM3*/+ sibs.

[b] *n* = number of male parents tested.

tives missing portions of the genetically defined centromere but had little effect on derivatives with intact centromeres. Mutations in the *ncd* and *klp3A* kinesin-like protein genes had the strongest dominant effects on centromere function. We demonstrated that derivative transmission could be sensitive to increased gene dosage, and we used increased nod^+ gene dosage to investigate the relationship of *nod* function to centromere activity. Taken together, these results indicate that screening for mutations affecting the transmission of centromere-defective derivatives will provide a general method to identify novel genes necessary for centromere function.

Heterozygous mutations in *trans*-acting genes reduce the transmission of a centromere-defective minichromosome: We hypothesized that genes interacting with the centromere could be identified from heterozygous mutations that reduce the transmission of centromere-defective *Dp1187* deletion derivatives. To test this hypothesis, we identified heterozygous mutations that reduced the transmission of the *J21A* derivative, which lacks approximately one-third of the 420-kb centromere region.

From a panel of mutant chromosomes with recessive effects on chromosome inheritance and a collection of *X* chromosome deficiencies, we identified eight chromosomes that dominantly reduced *J21A* transmission. Heterozygous *ncd*, *klp3A* and *Df(1)HA85* (a *nod* deletion) mutant chromosomes had strong effects. These mutations all identify kinesin-like protein genes. Kinesins have central roles in spindle assembly and chromosome motility (BARTON and GOLDSTEIN 1996). The dosage-dependent effects of *nod*, *ncd* and *klp3A* mutations may be particularly strong because the functions of spindle components depend on maintaining appropriate stoichiometry. Many examples of dose-dependent genetic interactions and nonallelic noncomplementation involve components of the cytoskeleton (HUFFAKER *et al.* 1987; FULLER *et al.* 1989).

Heterozygous *mei-S332*^{1} *ord*^{1}, *ord*^{1} and *Df(1)sd72b* chromosomes had weak effects on *J21A* transmission. Weak effects were seen as changes in *J21A* transmission of small magnitude (*e.g. Df(1)sd72b* and *mei-S332*^{1} *ord*^{1}) or more drastic loss of *J21A* transmission than normal with continued egg production (*ord*^{1} and *mei-S332*^{1} *ord*^{1}). Reduced transmission can result from mitotic or meiotic centromere instability, but decreasing transmission with continued brooding probably reflects chromosome loss in oogonial mitoses. A particularly strong example of a drop in *J21A* transmission with continued brooding was seen with *klp3A*. Small reductions in *J21A* transmission and slightly increased *J21A* transmission loss with brooding may be biologically significant, but very weak effects such as these are quite difficult to distinguish from the normal high variability in *J21A* transmission rates.

Interactions between heterozygous mutations and centromere-defective derivatives offer a way to screen for new genes important for chromosome segregation and show that this assay is robust enough to detect mutations with moderately strong effects. Strong reductions in *J21A* transmission like those seen with the *ncd*, *klp3A* and *nod* mutant chromosomes would be easily recognized, while small reductions like those seen with the *mei-S332*^{1} *ord*^{1}, *ord*^{1} and *Df(1)sd72b* chromosomes would not. Unfortunately, the strength of interaction does not necessarily reflect the importance of a gene to chromosome segregation: cellular protein levels may not be reduced enough by heterozygosity or the interacting region in the derivative may not be reduced enough in size to show strong effects on transmission, even though the protein is essential. Because dominant genetic interactions are idiosyncratic, the lack of effects in *l(1)zw10*, *pasc*, *grau* and *cort* heterozygotes cannot be taken as evidence of no role in chromosome segregation. In fact, ZW10 localizes to *Dp1187* (B. C. WILLIAMS, T. D. MURPHY, M. L. GOLDBERG and G. H. KARPEN, unpublished observations), and homozygous *l(1)zw10* mutations cause chromosome missegregation (WILLIAMS *et al.* 1992).

***Trans*-acting genes interact specifically with the centromere:** To determine whether *trans*-acting genes can interact specifically with the centromere, we assayed the effects of heterozygous *klp3A*, *ncd* and *mei-S332 ord* mutant chromosomes on the transmission of a series of *Dp1187* deletion derivatives. These mutant chromosomes reduced the transmission of partially stable derivatives missing portions of the centromere, but had little effect on the transmission of derivatives with intact centromeres. This pattern of interactions is clearly distinguishable from the pattern in *nod* heterozygotes, where extracentromeric regions were necessary for normal transmission (MURPHY and KARPEN 1995a).

Genetic interactions between heterozygous mutations and centromere-defective derivatives could reflect direct physical interactions between proteins and the centromere, or they could reflect indirect functional interactions. Cytological and biochemical experiments are necessary to distinguish these possibilities. The centromeric and extracentromeric interactions of *nod* likely correspond to the DNA binding of NOD protein: the *nod* gene sequence contains DNA binding motifs, NOD binds AATAT satellite repeats *in vitro*, and immunocytological observations show that NOD is distributed along chromosome arms (AFSHAR *et al.* 1995a,b). Indirect functional interactions may explain the genetic interactions of *ncd* and *klp3A* with centromere-defective derivatives, because NCD and KLP3A have identified roles in the spindle distinct from any role at the centromere. NCD bundles spindle microtubules and helps form the anastral meiotic spindle pole in females (HATSUMI and ENDOW 1992; MATTHIES *et al.* 1996). KLP3A is necessary for central spindle assembly during late anaphase of germ line mitoses and meiosis (WILLIAMS *et al.* 1995;

WILLIAMS *et al.* (1997). Neither KLP3A nor NCD has been seen to bind late metaphase or anaphase centromeres in immunocytological analyses (WILLIAMS *et al.* 1995; MATTHIES *et al.* 1996); however, light microscopic analyses do not have the resolution to determine whether NCD or KLP3A binds the centromere in addition to the spindle. Similarly, the genetic interactions of *ncd* and *klp3A* with centromere-defective derivatives are consistent with both direct and indirect functional interactions. Further biochemical studies are necessary to determine the exact cellular roles of NCD and KLP3A in centromere function. The centromere-specific interaction of the *mei-S332^{1} ord^{1}* chromosome may reflect direct or indirect interactions of sister chromatid cohesion proteins with the centromere.

The centromere contains *nod*$^{+}$ interacting regions: The effects of heterozygous *nod* mutations on derivative transmission demonstrated that *nod* interacts with extracentromeric regions (MURPHY and KARPEN 1995a). These genetic observations agree well with NOD localization along chromosome arms (AFSHAR *et al.* 1995a,b). Since tension is required for the stable attachment of kinetochores to microtubules (NICKLAS and WARD 1994), it was proposed that NOD-generated antipoleward forces stabilize kinetochore attachments during chromosome congression and segregation and during the recapture of detached chromosomes (THEURKAUF and HAWLEY 1992; MURPHY and KARPEN 1995a). What was not resolved by MURPHY and KARPEN (1995a) is whether *nod* interacts with the centromere in addition to interacting with extracentromeric regions.

Our experiments with increased *nod*$^{+}$ dosage suggest that *nod* interacts with the centromere. The partial rescue of *J21A* instability by increased *nod*$^{+}$ dosage demonstrates that *nod* interacting regions are still present in *J21A* and that increased *nod*$^{+}$ dosage compensates for deleting the centromeric region between the *J21A* breakpoint and the breakpoint in the smallest stable derivative, *10B*. What is the normal function of this region, and how is this rescue effected? One possibility is that the region interacts directly with NOD to help stabilize the attachment of the kinetochore to microtubules. The centromeric region between the *10B* and *J21A* breakpoints consists largely of AATAT repeats (LE *et al.* 1995; X. SUN, J. M. WAHLSTROM and G. H. KARPEN, unpublished results), and AFSHAR *et al.* (1995b) showed that NOD binds AATAT repeats with high affinity *in vitro*. These observations suggest a strong link between a specific segregation function and a specific centromeric sequence element. The second possibility is that the centromeric region between the *10B* and *J21A* breakpoints is required for kinetochore formation. Deletion of this region may result in a partially defective kinetochore, and increased NOD may compensate for this defect by stabilizing the partially unstable kinetochore attachments to microtubules. These two possibilities are not mutually exclusive; the region may be required for both NOD interactions and kinetochore formation. In fact, *J21A* is unstable in cell divisions where loss of *nod* function has no discernable effect (DAVIS 1969; CARPENTER 1973; ZHANG and HAWLEY 1990)—in males (MURPHY and KARPEN 1995b) and in somatic mitoses (K. COOK and G. KARPEN, unpublished results)—suggesting that AATAT repeats may be necessary in female meiosis for both NOD interactions and for other centromere functions such as kinetochore formation and sister chromatid cohesion.

It is currently unknown how poleward forces mediated by the kinetochore, antipoleward forces mediated by NOD, sister chromatid cohesion, and, perhaps, other less well-defined functions, such as specialized chromatin assembly, are integrated at the centromere. Each function could be associated with a physically distinct region of the centromere, or the functions could be physically interdigitated. Genetic interactions between *trans*-acting genes and *Dp1187* derivatives can potentially link all of the different centromeric sequence elements—AATAT satellite repeats, middle-repetitive and single copy sequences (LE *et al.* 1995), and AAGAG satellite repeats (X. SUN, J. M. WAHLSTROM and G. H. KARPEN, unpublished results)—to distinct centromere functions.

In conclusion, we have demonstrated that genetic interactions between altered gene dosage and centromere-defective *Dp1187* derivatives provide a means to identify *trans*-acting genes which interact specifically with the centromere. Screens for effects of heterozygous mutations on the transmission of centromere-defective derivatives hold promise for identifying novel genes necessary for centromere function. This approach targets chromosome segregation genes interacting with the centromere more effectively than previous screens in Drosophila, and allows one to screen mutations, such as lethals, whose effects on centromere function are difficult to assay in homozygotes.

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